analyzing the physical interaction of sox9 and the a SG promoter binding sites.

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Program/Abstract # 163
Generation of a sarcospan knock-down model in 3T3-L1 preadipocytes

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The sarcoglycan–sarcospan complex is composed of the transmembranal glycoproteins α, β, γ, δ, ε and ζ-SG, and the tetraspanin SSPN. Mutations in α, β, γ, and δ-SG are associated with autosomal recessive limb-girdle muscular dystrophies, while mutations in ε-SG causemyoclonus-dystonia syndrome. To this date no mutations in ζ-SG or SSPN have been associated with any disease or myopathy. However, transgenic SSPN knock-out mice present an 15–20% increment in body weight and 1.4–6.8 fold larger epididymal fat pad deposits compared to a wild type mice. Without wishing to be bound by theory, the increased weight gain and fat deposits are thought to reflect a disruption in metabolic signalling events. With the aim to explore this phenomenon we have generated a knockdown model in 3T3-L1 cells that will allow us to evaluate the probable function of this protein in lipid metabolism. 3T3-L1 preadipocytes maintained in DMEM containing 10% FBS were transfected with the a set of pSEC^{neo} expression vectors containing siRNA expression cassettes targeted to distinct regions in SSPN coding sequence. Inhibition of SSPN was analyzed by semiquantitative RT-PCR and indirect inmunofluorescense. Interferon mediated response was discarded through the analysis of the interferon gene target *oas1*. The most significant inhibition was obtained with the siRNA designed to recognize exon 1, which leads to a decrement of about 33.38% in protein levels. Future experiments involve the selection of stable transfectants in order to accomplish a better reduction in protein levels of SSPN.

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Program/Abstract # 164
Analysis of the Gro/Tle Co-repressors in pancreatic development

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Type 1 diabetes results from the auto-immune destruction of insulin producing β-cells in the endocrine pancreas. Understanding the transcriptional circuits regulating the specification and development of the hormone producing α and β cells of the endocrine compartment is vital for the development of new diabetes treatments. The Gro/Tles (Tle) are a family of transcriptional co-repressors that are involved in Notch and Wnt signaling and act as cofactors for the repression of target genes by engrailed homeobox 1 (eh1) domain containing transcription factors, such as members of the Nkx family. Many of the transcription factors that play key roles in pancreas development such as Nkx2.2, Nkx6.1, and Arx contain a Gro/Tle interaction (eh1) domain and act as context dependant transcriptional repressors. We detail here the co-expression of Gro/Tle proteins with these eh1 domain containing transcription factors during pancreas development. siRNAs targeting the Gro/Tles were used to assess their role in controlling cellular proliferation in vitro, as well as their expression in response to stimulation by Tgf-β, and Wnt signaling pathway ligands were determined. Finally we demonstrate biochemical interactions between the Nkx and Arx transcription factors and Tle3, and provide evidence for inter-family regulation of Tle3 function by Tle6. This project is funded by Genome Canada, Genome BC, and the BC Cancer Foundation.

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Program/Abstract # 165
Ptf1a binds to Area III, a highly conserved region of the Pdx1 promoter that mediates early pancreas-wide Pdx1 expression

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The Pdx1 transcription factor is expressed throughout the pancreas early, but mainly in insulin-producing β cells postnatally. Sequences in its 5′ region regulate this dynamic expression. The conserved regions Areas I and II (Pdx1PB) direct pancreatic endocrine cell expression, while an adjacent region (Pdx1XB) containing the conserved Area III directs transient β cell expression. Here we created Pdx1PBCre and Pdx1XBCre transgenic lines to lineage trace cells that activated these regions using a recombination reporter (R26R). Pdx1PBCre mediated exclusively endocrine cell recombination, while Pdx1XBCre mediated recombination throughout E10.5 pancreatic buds. A reporter transgene containing these conserved Areas is expressed throughout the E10.5 pancreas, and gradually becomes β cell restricted, similar to endogenous Pdx1.